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14. ABSTRACT

The NCCU/BBRI-Duke/Urology Partnership in Prostate Cancer Research was developed to promote Collaborative Training, Research, and Community Dialogue and Outreach among scientists from NCCU and Duke. During the first funding period, NCCU scientists, with the expertise of their Duke partners, have started five collaborative pilot projects. These projects have provided needed training for NCCU scientists in the development and approval of IRB protocols for research involving human subjects, and access to key core facilities and libraries at Duke. Some reagents important to the success of the projects have been developed including a mouse model of prostate cancer deficient in βarrestin 2 (TRAMP-βarr2$^{-/-}$) or 5-Lox (TRAMP-Alox5$^{-/}$). Several prostate cancer cell lines have also been adapted to the principal investigators laboratories for in vitro studies. Our collaboration has also led to the submission of three grant proposals: one to the USAMRMC and two to the National Institute of Health. The USARMC proposal entitled: "Association of the UGT2B17 Gene Deletion Polymorphism and the Incidence of Ovarian Cancer and Ethnicity" and the Export project entitled "Roles of Inflammation and Androgen Metabolism in Prostate Cancer Disparity" have been funded. The revised U54 application in partnership with UNC-Lineberger Comprehensive Cancer Center entitled "NCCU-LCCC Partnership In Cancer Research" is currently under review.

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Introduction:

Prostate cancer is the most common type of cancer found in American men, other than skin cancer. Prostate cancer is the second leading cause of cancer death in men. While one man in six will get prostate cancer during his lifetime, only one man in 35 will die of this disease. African-American men are more likely to have prostate cancer and more likely to die of it than are white or Asian men. The reasons for this are still not known. The long-term goal of the NCCU/BBRI-Duke/Urology Partnership is to develop innovative approaches for prevention, detection, and treatment of prostate cancer, through research, training and collaboration between these two institutions. Community outreach through one of the leading Historically Black Colleges and Universities (HBCU) in this country will eventually help address the issue of health disparity in Durham and the surrounding area of North Carolina, where there is a large population of African Americans.

Body:

Task #1: Create the Prostate Cancer Disparity Research and Training Center (PCDRT) and develop plan for training.

Task 1 has been accomplished. Several grants were submitted during the funding period of the NCCU/BBRI-Duke/Urology Partnership. Below is a list of the funded grants:

a) The U54 Cooperative Agreement grant submitted by Dr. Richardson in collaboration with the UNC Lineberger Comprehensive Cancer Center (LCCC) entitled "NCCU-LCCC Partnership in Cancer Research" has been funded for five years. The combined budget for the two institutions is ~12 million dollars. Two pilot projects (#3 and #4) supported by the NCCU/BBRI-Duke/Urology Partnership have been funded as full projects in the NCCU-LCCC Partnership in Cancer Research. The U54 grant is the largest research grant received by NCCU. (NCCU-LCCC Partnership in Cancer Research, NIH/NCI, CA156735)

b) Dr. Chen was awarded two grants as Principal Investigator:

   1. "Oxidative Stress in Alcohol-associated Oral Carcinogenesis" is funded as a full project in a collaborative partnership grant between NCCU and UNC-CH Bowles Center for Alcohol Studies ("Mechanisms of alcohol pathology", NIH/NIAAA U54AA019765).

   2. "Pig Model of Gastroesophageal Reflux Disease and Barrett's Esophagus" as a multidisciplinary research grant has been funded by the North Carolina Biotechnology Center (2011-MRG-1101).

c) Dr. Mukopadhyay was awarded two grants as Principal Investigator:

   1. "Canabinoid and alcohol interaction in neurogenesis" is funded as a full project in the collaborative partnership grant entitled "Mechanisms of alcohol pathology", (NIH/NIAAA U54AA019765).

   2. "CB2 Cannabinoid Receptor-mediated Regulation of Prostate Cancer Growth" under U54 Cooperative Agreement grant. (NCCU-LCCC Partnership in Cancer Research, NIH/NCI, CA156735)

d) Dr. Delores Grant was awarded one grant as Principal Investigator. She also submitted an RO1 grant that is currently under review.

1. "Association of the UGT2B17 Gene Deletion Polymorphism and the Incidence of Ovarian Cancer and Ethnicity". DOD Ovarian Cancer Research Program (OCRP) HBCU/MI Fellowship Award, (W81XWH-08-1-0406)

2. "Steroidic enzyme variant association with prostate cancer and hormones in Blacks". Submitted, NIMHD Health Disparities Research (R01) RFA-MD-12-001.

Task #2: Develop a core facility for the Collection of clinical samples and data.

The goals of task 2 have been accomplished. The core is integrated in the newly developed core facility entitled: **Diagnostics and Molecular Histopathology Facility** (DMHF). This new core lead by Dr. Xiaoxin Chen is supported by the U54 Cooperative Agreement grant (NCCU-LCCC Partnership in Cancer Research, NIH/NCI, CA156735)

Task #3: Develop 5 pilot studies focusing in the molecular, genetic socio-cultural aspects of prostate cancer incidence and disparities.

Below is the report from each pilot project along with the significance

Pilot Project #1: The UGT2B Gene Polymorphisms and its Association with Prostate Cancer Disparity
Investigator: Delores Grant, Ph.D. NCCU/BBRI
Collaborators: Cathrine Hoyo, Ph.D., Stephen Freedland, M.D., Joellen Schildkraut, Ph.D., Philip Febbo, M.D., Duke/Urology

A. Specific Aims

1. Determine whether there is an association between the *UGT2B17* deletion and *UGT2B15*^{D85Y} genotype in genomic DNA samples and prostate cancer risk using a case control study in African and Caucasian population.
2. Compare expression of the *UGT2B17* gene in RNA samples from the prostate cancer cases and controls and determine whether these also vary by race;
3. Quantify serum glucuronides of testosterone and testosterone metabolites among controls to determine association with 0, 1, or 2 copies of *UGT2B17* and;
4. Compare expression levels of the *UGT2B17* gene in prostate cancer tissue and normal margins utilizing tissue microdissection and immunohistochemistry.

B. Studies and Results

Aim 1. We have successfully accrued DNA, RNA, and serum samples and as of July 2010 began analysis of 347 cases and controls to determine the association between *UGT2B17* deletion and *UGT2B15*^{D85Y} genotype and prostate cancer. Our results showed that individuals homozygous for the minor Y allele of the *UGT2B15*^{D85Y} genotype had decreased risk (OR = 0.43; 95% CI = 0.2, 0.94)for prostate cancer (the major D allele is associated with risk) when adjusted for age, race, and waist circumference. Crude analysis of the main effect however showed that African Americans were more closely associated (p=0.15) than Caucasian. We cautiously interpret racial stratification at this point due to the relatively small number of cases. *UGT2B17* CNV was not associated with prostate cancer risk in this study population.

Aim 2. We successfully isolated total RNA from samples collected in PAX gene tubes and prepared and analyzed cDNA to determine whether the expression of the UGT2B17 gene was different in prostate cancer cases or controls or if the expression varied by race. Due to the

tissue specific nature of UGT2B17 expression, we were not able to successfully measure expression. We are continuing that analysis using immunohistochemical measurement of UGT2B17 in prostate tissue microarrays.

Aim 3. We were able to successfully measure the UGT2B17 conjugant, androstanediol glucuroide (AAG) in 147 serum samples and compare them to *UGT2B17* deletion, *UGT2B15*^{D85Y}, and *UGT2B7* polymorphisms. Notably, our results showed that *UGT2B17* deletion polymorphism was associated with AAG serum levels in Caucasians only (p=0.02) and that AAG levels for African Americans was again most closely associated with *UGT2B15*^{D85Y} polymorphism. These results must be interpreted cautiously and the study replicated in a larger sample size.

Aim 4. We are in the planning stage of determining UGT2B17 expression in prostate cancer tissue arrays. The microdissection and analysis of frozen tissue sections will be pursued with other funding.

C. Significance

These results suggest that the *UGT2B15*^{D85} polymorphism is associated with increased prostate cancer risk. The data also suggest an association between *UGT2B17* CNV and levels of androstane-3α,17β-diol-glucuronide in Whites. The results provide novel evidence that suggests that androstane-3α,17β-diol-glucuronide levels in Blacks maybe influenced differentially by UGT2B genotypes. However, due to the small size, a cautious interpretation due is warranted. This study contributes to the elucidation of the genomic differences that are associated with risk for prostate cancer but that also those that explain the underlying biological differences between populations in order to genetically decipher reasons for health disparities.

D..
We are in the process of finalizing a manuscript that will be submitted for publication shortly. In addition, we are continuing our study by pursuing competitive research funding and have recently submitted an RO1 towards that goal.

Pilot Project #2: Role of β-arrestins in prostate cancer development and its contribution to Prostate Cancer Disparity

Investigator: M. Ricardo Richardson, PhD. NCCU/BBRI
Collaborators: Judd Moul, M.D., Duke/Urology

A. Specific Aims

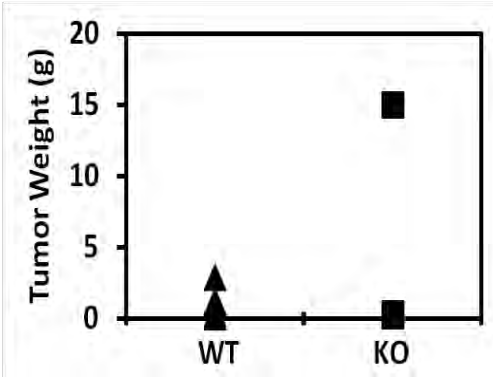
1. To determine whether the level of expression of βarr1 and/or βarr2 are elevated in prostatic tissues from African American Men (AAM) relative to Caucasians American men (CAM).
2. To develop the TRAMP mouse model of prostate cancer in mice deficient in either βarr-1 or βarr-**2.**

B. Studies and Results

Using several prostatic cell lines (RWPE-1, PC3, LNCaP, E006AA and MDAPCa2b) we have shown that these cells express βarr1 and to a lesser extent βarr2. Gene expression analysis of βarr1 correlates with level of protein found in these cells. Interestingly, while βarr2 is expressed at higher levels in both Caucasian American (CA) derived cells lines (RWPE-1, PC3 & LNCaP) than in the African American (AA) derived cell lines (MDA PCb2a & E006AA, western blot shows that the protein is much more abundant in both AA prostate cancer cell lines than their CA counterparts. Peripheral blood analysis of CA and AA prostate cancer patients showed significant increases in βarr1 and βarr2 expression relative

to healty controls (HC). Although βarr1 expression levels were higher than βarr2, no significant difference was found in CA versus AA patients. Preliminary results on the growth of prostate tumors of TRAMP mice deficient in βarr2 expression ($TRAMP^{+}$- $\beta arr2^{-/-}$) showed no significant effect in tumor size relative to control animals ($TRAMP^{+}$- $\beta arr2^{+/+}$). Taken together, these data indicate that βarr2 likely play a limited role in prostate tumor development and the disparity between black and white men.

In light of these results we have generated a TRAMP-$\beta arr1^{-/-}$ mouse model to test *in vivo* the hypothesis that $\beta arr1^{-/-}$ modulate prostate tumor progression and metastasis.



βarrestin-2 deficiency has no significant effect in tumor development in TRAMP mice. 6 month old $TRAMP^{+}$/barr2 KO and $TRAMP^{+}$/barr2 WT mice were sacrificed and the tumor weight obtained. WT, n=5: KO. n=2. P=0.16.

Significance

The data obtained thus far have shown that a) βarr1 protein is more abundant in prostate cancer cell lines from both CA and AA than βarr2; b) expression of βarrestin 1 and 2 in white blood cells does not differ in black and white prostate cancer patients and; c) suppression of βarr2 expression in the TRAMP mouse model has no significant effect in prostate tumor growth**.**

Pilot Project #3: Role of 5-Lipoxygenase in Clinical Outcome of African American and Caucasian Prostate Cancer Patients

Investigator: Xiaoxin Chen, M.D., Ph.D., NCCU/BBRI
Collaborator: Leon Sun, M.D., Duke/Urology

A. Specific Aims

To determine whether expression and regulation of *Alox5* and *blt1* by promoter methylation and polymorphism may contribute to prostate cancer disparity between African American and Caucasian men.

B. Studies and Results

1. We have performed immunohistochemical staining of 5-lipoxygenase (5-Lox) on paraffin sections of 150 cases of African American prostate cancer and 150 cases of Caucasian American prostate cancer. Initial analysis has shown significant increase of 5-Lox in cancer versus normal tissue. However, we failed to detect any significant difference between African American and Caucasian American samples.

2. We have not yet reached a solid conclusion regarding the potential role of promoter methylation in regulating expression of these two genes. We examined the effect of 5-aza-2'-deoxycytidine (a DNA demethylating agent) on the expression of 5-Lox and BLT1 in two human prostate cancer cell lines (LNCaP, PC3) and two human normal prostate epithelial cell lines (PrEC1 and PrEC2). Both RT-PCR and Western blotting failed to show dramatic up-regulation of these genes. It suggested that expression of these two genes is not mainly regulated by promoter methylation.

3. One animal experiment is ongoing to determine whether knockout of *Alox5* may reduce tumorigenesis in TRAMP mice. We have two groups of mice, TRAMP/$Alox5^{+/+}$ mice and TRAMP/$Alox5^{-/-}$ mice. Survival curves of these mice are shown as follows. Since our TRAMP/$Alox5^{+/+}$ mice are still relatively young, no significant difference can be observed before the age of 20 weeks.

C. Significance

This project is specifically designed to address one significant issue in health disparity of prostate cancer, why African American men have a higher risk of developing prostate cancer and poorer clinical outcome than Caucasian American men. However, experimental data do not agree with our original hypothesis.

Pilot Project #4: Anandamide-mediated Regulation of Prostate Cancer Cell Proliferation and Angiogenesis in African Americans

Investigator: Somnath Mukhopadhyay, PhD, NCCU/BBRI
Collaborator: Judd Moul, MD, Duke/Urology

A. Specific Aims

1) To define the role of CB1 and CB2 cannabinoid receptors in endocannabinoid methanandamide-mediated cell proliferation and androgen receptor expression in EA006AA African American prostate cancer cells.

2) Characterization of anandamide-mediated regulation of matrix metalloprotease (MMP) activity in E006AA prostate cancer cells.

B. Studies and Results

This project has addressed a health disparity of prostate cancer. Specifically, this project focuses on the effect of CB2 receptor activation on cell proliferation, viability and cell motility of non-African-American prostate cancer cells and African-American prostate cancer cells. Collectively the results from this pilot study suggests that CB2R-FAK-MMP signaling axis is functional in E006AA African-American prostate cancer cell lines. Further, results from this study has identified CB2 receptor as a novel target in the regulation of prostate cancer African-American prostate cancer cells. **The Cumulative report of research findings are described below:**

a) In African American prostate cancer cell line (E006AA) the level of CB2 receptor (CB2R) expression is higher than normal prostate epithelial cells (PrEC).

b) CB2 receptor activation produced significant decrease in LNCaP prostate cancer cell viability but did not produced any significant change in non-malignant PrEc cell viability suggesting that activation of CB2 receptor reduce cell viability of malignant cells with no effect on normal cells.

c) CB2 receptor activation led to significant decrease in E006AA cell proliferation and cell migration of in a dose-dependent manner and CB2R antagonist (SR144528) inhibited CB2R agonist-induced decrease in E006AA cell migration and proliferation.

d) Under *in vitro* cell culture condition without any treatment (Control) LNCaP cell and E006AA cells exhibit a significant FAK phosphorylation at Tyr397; Treatment with CB2 receptor agonist produced a significant dephosphorylation at as early as 5 min of treatment and produced more robust dephosphorylation when tested at 15 min.

e) When LNCaP and E006AA cells were pre-treated with CB2 receptor antagonist SR1445282, FAK dephosphorylation at Tyr 397 was inhibited.

f) CB2 receptor activation significantly decreased secreted PSA level in LNCaP and E006AA cells. However, we did not find any significant difference in the percent decrease between LNCaP and E006AA cell lines.

g) CB2 receptor activation reduced FBS-induced cell migration in E006AA and LNCaP cells in a dose-dependent manner and this effect was reversed by CB2 receptor antagonist SR144528. We tested the efficacy of several CB2 receptor agonists (HU308, JWH 015, AM1241) for inhibition of cell migration in the above mentioned cells lines but no difference in IC_{50} was found between the cell lines for these CB2 receptor agonists.

h) CB2 receptor agonist treatment for 5 min produced activation of RhoA in 5 min but then it comes back to control level suggesting that CB2 receptor activation increased RhoA signaling in a time-dependent manner.

i) CB2 receptor activation inhibits MMP activity in E006AA cells. This decrease in MMP2 and/or MMP9 activity can be correlated with CB2-receptor-mediated decrease in cell migration. Further we found that transient overexpression of dominant-negative FAK (FRNK) in E006AA cells blocked CB2 receptor-mediated decrease in MMP activity. This suggests that activation of CB2 receptor regulates MMP activity in E006AA cells in a FAK dependent manner.

Significance

This project is addressing a health disparity of prostate cancer. Specifically, this project focuses on the effect of CB2 receptor activation on cell proliferation, viability and cell motility of non-African-American prostate cancer cells and African-American prostate cancer cells. The results from this study will be helpful to a) determine the differential effect of CB2 receptor activation in the regulation of prostate cancer in non African-American prostate cancer cells and African-American prostate cancer cells; b) validate CB2 receptor as a novel drug target for the regulation of prostate cancer. Thus, the outcome of this project may identify novel target for therapeutic intervention against prostate cancer

Pilot Project #5: Feasibility of Endurance Exercise Training on Cardiovascular Risk Factors Following Radical Prostatectomy among Men with Localized Prostate Cancer: A Community-Based Intervention

Investigators: Dave Tulis Ph.D., NCCU/BBRI

Collaborators: Catherine Hoyo, Ph.D., Lee Jones, Ph.D., Stephen Freeland, M.D., Duke/Urology

A. Specific Aims

1. To determine the effects of home-based endurance exercise training on exercise capacity following radical prostatectomy among with men with localized prostate cancer.
2. To assess the changes in other markers of CVD (i.e., lipid profile, blood pressure, fasting insulin, C-reactive protein, and weight status).
3. To explore the potential differential effects of exercise training between white and black American prostate cancer patients on specific aims 1 and 2.

B) Studies and Results

As mentioned in the last progress report, this project was terminated. The data generated led to the publication of the manuscript entitled “Exercise is Associated with a Reduced Risk of Prostate Cancer in a Cohort of Veterans Undergoing Prostate Needle Biopsy” (see Reportable outcomes)

Task #4: Training Determine the effects of home-based endurance exercise training on exercise capacity following radical prostatectomy among with men with localized prostate cancer.

As indicated in last year progress report, the funds for this project were rebugeted to hire a Research Associate 100% at Duke to help with the collection of the samples

Key Research Accomplishments: N/A

Reportable Outcomes:

We have consented and have questionnaire data on 1164 prostate cancer cases, healthy controls, biopsy negative controls. We also have approximately 400 healthy control serum samples to further pursue studies on genotype association with AAG levels.

Submitted or Published Manuscripts

Antonelli, J., Jones, LW, Banez, LL, Thomas, J-A, Anderson, K, Taylor, LA, Gerber, L, Crowe, N, Anderson, T, Hoyo, C, Grant, DJ, Freedland, SJ. 2009. Exercise is Associated with a Reduced Risk of Prostate Cancer in a Cohort of Veterans Undergoing Prostate Needle Biopsy. J Urology. 182: 2101-2.

Grant, DJ, Moorman, PG, Akushevich, L, Palmieri, RT, Bentley, RC, Schildkraut, JM. 2010. Primary Peritoneal and Serous Ovarian Cancer: An Epidemiological Comparative Analysis. Cancer, Causes, and Control. Cancer, Causes, and Control. 21:991-8

Williams, CD, Brian Whitley, B, Hoyo, C, Grant, D, Iraggi, J, Newman, K, Gerber, L, Taylor, L, McKeever, M, and Freedland, SJ. 2011. A high ratio of dietary n-6/n-3 polyunsaturated fatty acids is associated with increased risk of prostate cancer. Nutrition Research. 31(1):1-8

Moreira, DM, Anderson, T, Gerber, L, Thomas, J-A, Bañez, L.L., McKeever, M.G., Hoyo, C, Grant, D, Jayachandran, J and Freedland, S.J. 2011. The Association of Diabetes Mellitus and High-Grade Prostate Cancer in a Multiethnic Biopsy Series. Cancer, Causes, and Control. May 12 [Epub ahead of print].

Vladimir Poltoratsky, Inneke Johnson, Shalini Jha, Jayprakash Saha, Sahariar Koochekpour and Somnath Mukhopadhyay Targeting CB2 cannabinoid receptor for regulation of prostate cancer growth (submitted)

Conclusion:

The NCCU-Duke Collaborative Center in Prostate Cancer has allowed NCCU scientists to interact with investigators of the Duke Prostate Center to develop collaborative projects focusing in prostate cancer disparity. Although the funding was limited, we were able to publish several manuscripts in collaboration with the Duke partners. The data obtained has also led to submission of several successful grants to NIH and other funding agencies. Our biggest accomplishment thus is the successful submission of U54 partnership grant. It has provided funding to continue some of the pilot projects supported by the DOD partnership including the development of a new core facility entitled: **Diagnostics and Molecular Histopathology Facility** (DMHF). Overall the HBCU collaborative NCCU/BBRI-Duke/Urology Partnership has been a success.